Results: Coculture of the U937 cells with synovial fibroblasts resulted in differentiation of U937 into monocytes, as evidenced by FACS indicating that they were CD14+. Over 7 days, the cytokine and chemokine levels in the media of these cocultured cells with cartilage explants approached levels similar to those reported for OA synovial fluid, including low levels of Il-1β and TNFα. GAG release was observed in co-cultures stimulated with Il-1β, uPA, or Fn-f, with the most severe effects in the Il-1β-treated group (Figure). However, the concentration of Il-1β used in the control samples, typical of concentrations used by others for release of GAGs from cartilage disks in vitro, is far greater than the concentration reported in OA synovial fluid. Pretreatment with Il-1ra was able to inhibit the loss of GAG from the cartilage in the Il-1β stimulated samples, but did not block Fn-f-stimulated GAG release in the co-culture system, and had a small effect on uPA stimulated GAG release.
Conclusions: An in vitro model of cartilage degradation has been developed utilizing a co-culture of human synovial fibroblasts with differentiated U937 cells, in the presence of bovine cartilage explants. The interaction of these cell types generated a cytokine profile similar to OA synovial fluid, and stimulation of this system with uPA or Fn-f led to cartilage degradation (GAG loss). Our ongoing work will continue to explore the roles of various cytokines and the molecular mechanisms through which they influence cartilage degeneration in this in vitro joint model system, and their potential relation to OA disease progression.

Matrix Biochemistry

444 OSTEOARTHRITIS AND AGE RELATED CHANGES IN AGGRECAN AND COLAGEN IN HUMAN HIP CARTILAGE

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Purpose: To confirm that previously reported changes in collagen and GAG content from knee OA cartialge also occur in hip OA and that these changes are different from those in reference cartilages. We also examined the relationships between total collagen, % denatured collagen and GAG content and with age.
Methods: 18 OA (aged 45 to 81, 11 women) and 26 reference (aged 55 to 99 years, 20 women) femoral heads were collected at hip replacement surgery, due to OA or femoral neck fracture. OA cartilages were taken from sites where it was possible to sample full-depth cartilages whereas reference cartilages were taken from the superior most loaded areas. Cartilages from the same site were examined for routine histology and immunohistochemistry. Each sample was divided into two parts that were either, digested with 1 mg/ml α-Chymotrypsin in 50 mM tris containing the proteinase inhibitors to extract denatured collagen, or with papain at 60C for 18 h (1−2 μl of papain/10 mg wet weight tissue) in 0.1 M tris-HCl, pH 7.2, containing 0.01 M disodium EDTA and 0.005 cysteine-HCl to enable GAG analysis. The Chymotrypsin supernatants (containing cleaved collagen) and residues (containing intact collagen) were hydrolysed in 12N and 6N HCl, respectively, at 110ºC overnight and then dried. Subsequently the samples were dissolved in 500 μl of distilled water and clarified using an equal amount of charcoal and AG-1 X8 anoin exchange resin. The amount of hydroxyproline (Hyp. μg/mg wet weight) was measured colorimetrically at 550 nm. The values from the assay were compared to those from a standard curve prepared with L-4-Hydroxyproline. Amounts of hydroxyproline were expressed either as total collagen or % degradation. The papain-digested samples were assayed for GAG by a commercially available kit using dye-precipitation of sulphated GAGs with Alcian blue (www.wieslab.com).
Results: The reference group was older than the OA group ($p < 0.001$). Collagen content was 23% lower in OA than reference cartilage ($p = 0.002$). % Denatured collagen was 2.5 times higher in OA than that of reference cartilages ($p = 0.001$).
% Denaturation was inversely related to collagen content in OA cartilage ($r = -0.78$, $p = 0.000$) but not in the reference cartilage ($p = 0.5$).
GAG content was similar in reference and OA cartilages. GAG content was inversely related to % denatured collagen in OA and reference samples; lower GAG was present in samples with more denatured collagen. Amounts of collagen and GAG and % denatured collagen were not related to patients' age. The Mankin and immunohistochemistry grades, higher in OA than in reference samples ($p < 0.001$), were not related to % denaturation, collagen or GAG content.
Conclusions: The present study provides new perspectives of the relationships between the two main molecules, collagen and aggrecan, in OA and reference articular cartilage. Similar collagen damage in hip OA cartilage as previously shown in knee OA cartilage suggest similar OA degradative mechanisms of collagen in different joints. With respect to age, our study shows similarities between OA and reference cartilage. The lack of relationships between molecule contents and age may suggest that subjects age is not related to disease activity or suseptibility. Rather we believe that exposure time to a risk factor is of importance. The increased degree of denatured collagen and the inverse relationship between % denatured collagen and collagen content in OA compared to reference cartilage indicates mechanisms of progressiveness in disease that are not activated in normal ageing. Therapeutic interventions in progressive hip and knee OA may include means to control cartilage collagen content using inhibitors that decrease collagen degradation as well as treatments that increase cartilage GAG content in order to improve cartilage quality.

445 P38 MAPK IS AN ESSENTIAL UPSTREAM SIGNALLING PATHWAY IN PROTEOLYTIC CARTILAGE DEGRADATION

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Purpose: Matrix Metalloproteases (MMP) and aggrecanases are essential players in cartilage degradation. However, the signaling pathways involved that results in MMP and aggrecanase synthesis and activation are not well understood. We investigated whether the p38 MAPK was involved in processes leading to collagen type II degradation in catabolic stimulated articular cartilage.
Methods: Bovine articular cartilage explants were isolated from stifle joints from slaughtered cows 1.5−2 years of age. The explants were cultured serum-free in Dulbecco's Modified Eagle's Medium in the presence of 10 ng/ml Oncostatin M (OSM) and 20 ng/ml Tumor Necrosis Factor-α (TNF) with or without the specific inhibitors of p38 MAPK SB-203580 or SB-202190, respectively, in doses of 0.1, 1 or 10 μM for 21 days with change of conditioned medium every 2−3 day. Cartilage explants cultured in medium alone and metabolically inactivated explants were used as control. Cell viability was followed by the Alamar blue colorimetric assay. Total cartilage proteoglycans were isolated from the cultured cartilage explants after the culture period by 4 M GuHCl extraction for 48 h. Collagen type II degradation was analyzed by the CTX-II assay and by hydroxyproline quantification. MMP activity was assessed in the conditioned medium using the Mca-Pro-Leu-Gly-Leu-Dap(Dnp)-Ala-Arg-NH_2 MMP substrate with a quencher and a highly fluorescent end, and the cleavage was followed by quantification of the fluorescence.
Results: Stimulation of cartilage degradation by OSM + TNF resulted in a 100-fold induction of CTX-II release ($p < 0.01$), compared to vehicle treated and metabolic inactive controls. The total levels of proteoglycan in the explants decreased by 50%, $p < 0.0001$ in the OSM + TNF treated cartilage compared to vehicle control, but the p38 inhibitor SB-203580 was able to retain the levels of proteoglycans by 35%, $p < 0.01$, compared to vehicle control. Exposure of the articular cartilage explants to OSM + TNF, and the specific p38 MAPK inhibitors SB-203580 or SB-202190, resulted in a dose-dependent complete abrogation of MMP mediated collagen type II degradation (CTX-II). This was in alignment to the quantification of hydroxyproline levels, ($p < 0.01$). In support, p38 MAPK inhibition resulted in a dose-dependent abrogation of MMP activity measured by the fluorescence assay. In addition, no toxic effect was observed on cell viability.
Conclusions: We found that the inhibition of p38 MAPK abrogated proteolytic cartilage degradation by blocking MMP synthesis and activity. These results suggest that pathways resulting in less p38 signaling may be potential options for indirect modulation of MMP expression and activity. This may be important for the discovery of new potential OA treatments.

446 DETECTION OF THE EARLY CHANGE OF THE CARTILAGE DEGENERATION WITH FTIR AND ULTRASOUND EVALUATION

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Purpose: Early diagnosis of osteoarthritis is important, although the diagnostic method is not fully established yet. It is well known that